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Immune Response of Pigs to Inactivated Foot-and-Mouth Disease Vaccines

Response to DEAE-Dextran and Saponin Adjuvanted Vaccines

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SUMMARY. The immune response of pigs to inactivated DEAE-dextran and saponin adjuvanted foot-and-mouth disease (FMD) vaccines differed from their response to emulsion vaccines. A single dose of a DEAE-dextran vaccine stimulated reasonable serum neutralizing antibody concentrations which reached their peak at 14 days after vaccination and then decreased. Duration of immunity was short and lasted from 1 to 3 months. A single dose stimulated the appearance of IgG antibodies. A good anamnestic response resulting in a much longer duration of immunity followed secondary vaccination at between 1 and 3 months after primary vaccination. A single dose of a saponin vaccine gave a true IgM primary response with peak antibody concentrations at 7 days after vaccination. By 21 days no detectable serum antibodies were present, but the animals were capable of giving a moderate anamnestic response to a second inoculation at this time which resulted in the appearance of IgG antibodies. It is suggested that the pig requires repeated stimulation with inactivated FMD antigen for an adequate level of immunity to be maintained. This occurs following vaccination with emulsion vaccines but does not occur in convalescent pigs or following inoculation with DEAE-dextran or saponin vaccines.

THE RESPONSE OF pigs to inactivated FMD vaccines has been reviewed by Mussgay & Wittmann (1968). Up to that time most of the work had been done with vaccines containing aluminium hydroxide or saponin as the adjuvant. These vaccines were consistently poor and increasing the antigen dose or the use of different inactivants did not improve their potency. This and the short duration of immunity of convalescent pigs led these authors to conclude that it was unlikely that an inactivated vaccine effective in pigs would be developed. Since that time it has been shown that the emulsion vaccines stimulate a good immunity in pigs and more recently Wittmann *et al.* (1970) have reported that the use of DEAE-dextran adjuvanted vaccines resulted in almost total protection of pigs exposed to natural contact infection 12 weeks after vaccination. The purpose of this report is to compare the serological responses obtained and the class of antibody present following primary and secondary vaccination with DEAE-dextran and saponin adjuvanted vaccines under conditions similar to those reported for the emulsion vaccines (Anderson *et al.*, 1971).

MATERIALS AND METHODS

Virus Strains

Two virus strains were used:

Type and subtype	*Strain*
C	Noville
O_1	Swiss 1/66

Antigen Production and Assay

This has been described in our previous paper (Anderson *et al.*, 1971).

Vaccine Formulation

DEAE-Dextran Vaccines

(i) *Preparation of the Adjuvant.* In all the experiments with the exception of those carried out to determine the effect of different doses of DEAE-dextran on the immune response, a standard dose of 100 mg. DEAE-dextran in 2 ml. of vaccine was used. A solution containing 100 mg./ml. was prepared in 0·25M tris-HCl buffer of pH 8·2. This was autoclaved and the pH measured to ensure it was 7·6–7·8. When levels of 250 mg. and 500 mg. DEAE-dextran were included in the 2 ml. vaccine dose they were prepared in 0·5M tris-HCl buffer of pH 8·2.

(ii) *Formulation of the Vaccine.* To the adjuvant solution was added an equal volume of the antigen suspension at pH 7·6. All the vaccines were given subcutaneously in a 2 ml. volume.

Saponin Vaccines

A solution containing saponin* 5 mg./ml. was prepared in phosphate buffered saline (PBS). To this was added an equal volume of the antigen suspension. A 2 ml. volume was administered.

Animal Experiments

Large White cross Landrace pigs of between 30 and 40 kg. bodyweight were used. Blood samples were collected at frequent intervals and assayed for serum neutralizing antibody concentrations by the cell metabolic inhibition test (Martin & Chapman, 1961) in all the experiments except that to determine the effect on the immune response of increased amounts of DEAE-dextran. Here, because suitable cell cultures were not available, neutralizing antibody concentrations were assayed in 5- to 7-day-old mice using 3-fold serum dilutions and a fixed virus dose of 100 mouse ID_{50}, the 50% end-point being calculated by the method of Reed & Muench (1938). Antibody concentrations are quoted as the group geometric mean for each group of pigs. Results are expressed as the $\log_{10}$ of the reciprocal of the serum end-point dilution. Where animals were challenged, the method used was to inoculate 100 pig ID_{10} of homologous virus into the heel of 1 foot and observe the pigs for signs of generalized lesions (Burrows, 1966).

Examination of Antibody Class

This was done as previously described (Anderson *et al.*, 1971).

RESULTS

DEAE-Dextran Vaccines

Antigen Threshold

(i) *Type C Virus (C Noville).* The inactivated antigen concentrate used contained 1400 c.f.u./ml. of which an estimated 38% consisted of the 140S component. Using an Eagle's + tryptose phosphate broth serum-free medium as the diluent, a dilution of the antigen concentrate containing 175 c.f.u./ml. was prepared. Four-fold serial dilutions of this suspension were made to produce an antigen range of 175 to 3 c.f.u./ml. To each antigen dilution was added an equal volume of adjuvant containing DEAE-dextran 100 mg./ml. Each vaccine was given to groups of 8 pigs in a 2 ml. volume. Humoral antibody concentrations were assayed 7, 14, 21 and 28 days after inoculation and all the pigs were challenged at 28 days after inoculation. Antibody concentrations reached a peak at 14 days after inoculation (Fig. 1a) and then decreased rapidly. There was no response to the smallest antigen dose. At 14 days after inoculation antibody titres were similar in all but the group that received the smallest antigen dose, but thereafter antibody concentrations fell more slowly in the group that received the largest antigen dose. The slope of the dose response regression line at 28 days after inoculation (Fig. 1b) indicates that increasing the antigen

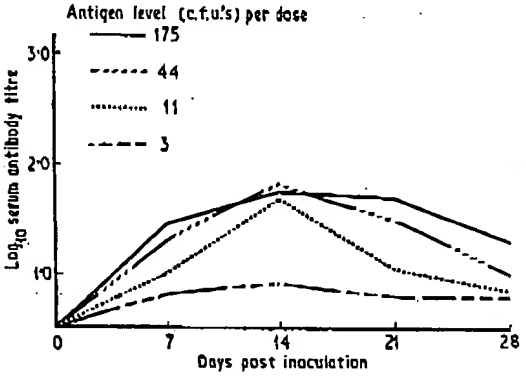

FIG. 1a. Serum antibody response to type C DEAE-dextran vaccine.

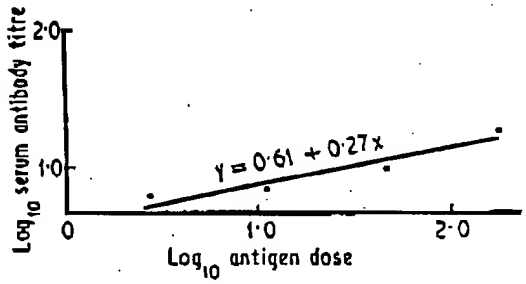

FIG. 1b. Dose response regression analysis of antibody concentration at 28 days after vaccination.

* Food Industries Ltd, Cheshire.

TABLE I
LEVELS OF PROTECTION IN GROUPS OF PIGS VACCINATED WITH TYPE C DEAE-DEXTRAN VACCINES AND CHALLENGED WITH 100 PIG ID_{50} OF HOMOLOGOUS VIRUS

	Antigen (c.f.u.) per dose			
	175	43·75	10·9	2·75
No. protected/total	8/8	6/8	3/7	2/8

dose would result in an improved immune response. The pigs were challenged at 28 days after inoculation and the results are shown in Table I.

This vaccine was found to have a PD_{50} value of 12·8 c.f.u. total antigen or 4·9 c.f.u. in terms of 140S component. This compared with values of 0·33 c.f.u. total antigen or 0·13 c.f.u. in terms of 140S component for the double emulsion vaccine prepared with the same batch of antigen concentrate (Anderson *et al.*, 1971). The DEAE-dextran vaccine was not as potent as the double emulsion vaccine and in addition produced an antibody response curve of a different shape with antibody concentrations not being maintained beyond 14 days after inoculation.

(ii) *Type O Virus* (*O Swiss* 1/66). The inactivated antigen concentrate used contained 1620 c.f.u./ml. of which an estimated 30% consisted of the 140S component. A dilution of this concentrate was prepared to contain 200 c.f.u./ml. Four-fold serial dilutions of this suspension were prepared to cover an antigen range of 200 to 3 c.f.u./ml. To each antigen dilution was added an equal volume of adjuvant containing DEAE-dextran 100 mg./ml. Each vaccine was given to groups of 6 pigs in a 2 ml. volume. Humoral antibody concentrations were assayed weekly up to 28 days after inoculation when the animals were challenged. The antibody response curves (Fig. 2a) show that peak antibody concentrations occurred at 14 days after inoculation and then decreased sharply so that at 28 days after inoculation all the antibody concentrations were small.

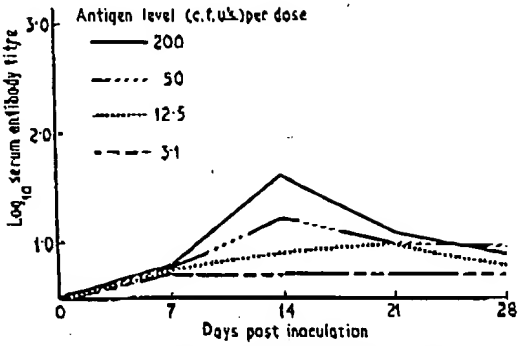

FIG. 2a. Serum antibody response to type O DEAE-dextran vaccine.

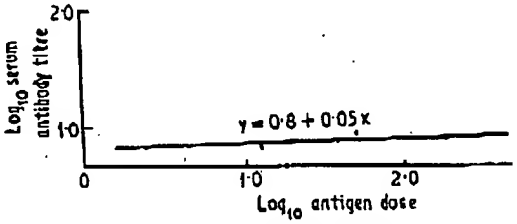

FIG. 2b. Dose response regression analysis of antibody concentrations at 28 days after vaccination.

In contrast to the type C vaccine there was little correlation between antigen dose and antibody response at 28 days after inoculation (Fig. 2b) but there was a marked correlation at 14 days after inoculation. The pigs were challenged at 28 days after inoculation and the results are shown in Table II.

The PD_{50} value of this vaccine was found to be > 120 c.f.u. total antigen. This compared with a value of 0·2 c.f.u. total antigen for the double emulsion vaccine prepared with the same antigen concentrate (Anderson *et al.*, 1971).

TABLE II
LEVELS OF PROTECTION IN GROUPS OF PIGS VACCINATED WITH TYPE O DEAE-DEXTRAN VACCINES AND CHALLENGED WITH 100 PIG ID_{50} OF HOMOLOGOUS VIRUS

	Antigen (c.f.u.) per dose			
	200	50	12·5	3·1
No. protected/total	2/4	0/5	2/5	0/3

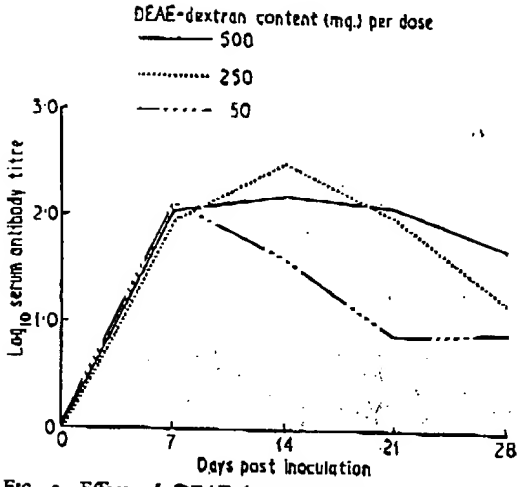

FIG. 3. Effect of DEAE-dextran dose on the immune response.

Effect on the Immune Response of Increased DEAE-Dextran Doses. Using a type O antigen, vaccines were prepared containing in each 2 ml. dose 200 c.f.u. total antigen or 48 c.f.u. in terms of the 140S component and either 50, 250 or 500 mg. DEAE-dextran. Each vaccine was administered to a group of 6 pigs and blood samples were collected at weekly intervals. Antibody concentrations were assayed in 5- to 7-day-old unweaned mice and the results are shown in Fig. 3.

A quantitative response to this adjuvant was shown as titres in the group receiving 50 mg. reached an early peak at 7 days after inoculation and then decreased sharply while those in the 250 mg. and 500 mg. groups continued to increase to 14 days after inoculation and then decreased slowly, more so in the 500 mg. group. No undesirable tissue reaction to any of these vaccines was found.

Duration of Immunity. A group of 10 pigs was given a single dose of a type C vaccine containing 175 c.f.u. total antigen and 100 mg. DEAE-dextran. Antibody concentrations were measured at intervals up to 180 days after inoculation. The result is shown in Fig. 4 which indicates that concentrations were never high and by 56 days after inoculation had fallen below the average level of 1·2

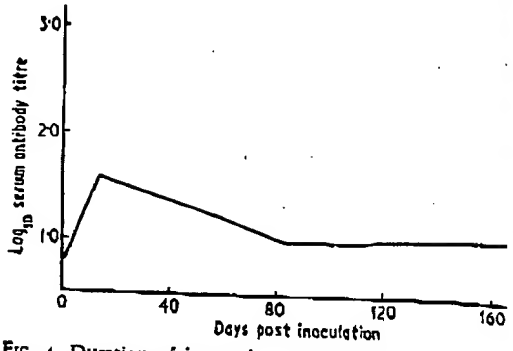

FIG. 4. Duration of immunity to type C DEAE-dextran vaccine.

known to correlate with 50% protection against a needle challenge with 100 pig ID_{50} of homologous virus (unpublished results).

Anamnestic Response. Two groups of 4 pigs each were given a dose of the type C vaccine containing 175 c.f.u. total antigen and 100 mg. DEAE-dextran. One group was then given a second dose of the same vaccine 28 days later and the other group a second dose 84 days after the primary dose. Blood samples were collected at intervals and antibody titres assayed. A good anamnestic response was observed in both groups (Fig. 5) but in order to maintain an adequate level of immunity a second dose would need to be given within 2 to 3 months of the first dose. This would result in an acceptable level of immunity for at least 6 months for this strain of virus.

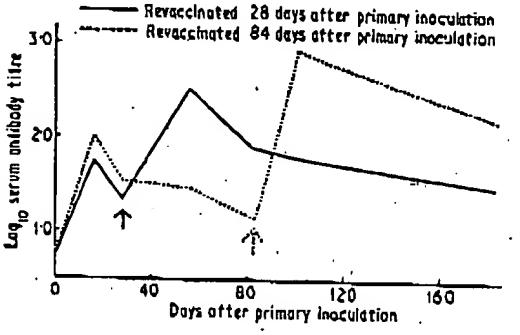

FIG. 5. Anamnestic response to a secondary inoculation of a type C DEAE-dextran vaccine given at 28 or 84 days after a primary dose of the same vaccine.

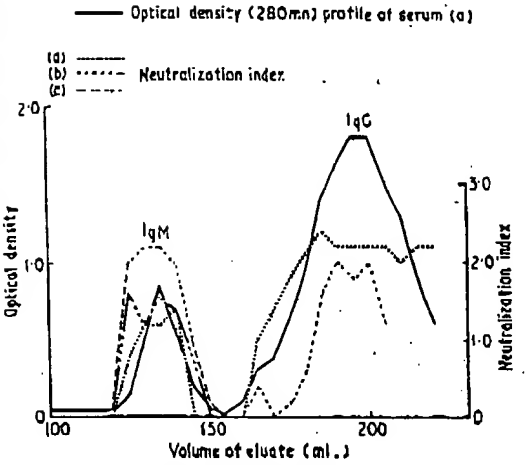

FIG. 6. Neutralizing antibody levels, expressed as the 'neutralization index' (NI) of Sephadex G-200 fractions of sera collected from 3 pigs 28 days after vaccination with a type C DEAE-dextran vaccine.

Antibody Class at Intervals Following Vaccination. The class of antibody present 28 days after primary vaccination in 3 pigs that received a type C vaccine containing 175 c.f.u. total antigen was examined.

The G-200 profiles are shown in Fig. 6. Two animals, which had serum antibody titres of 1·80 and 1·05 respectively as measured by the cell metabolic inhibition test, were found to have both IgM and IgG present. The third animal with a lower serum antibody titre of 0·90 had only IgM present. This indicated that higher antigen doses maintained serum antibody for a longer period and this was associated with the change from IgM to IgG production. Where this change has occurred a good secondary response may be expected.

Saponin Vaccines

A single dose of a saponin vaccine resulted in a true primary response (unpublished results). There was an initial increase in circulating neutralizing antibodies which reached a peak at 7 to 10 days after vaccination and then decreased rapidly so that by 21 days after vaccination no antibody was detectable. All the antibody produced was 19S immunoglobulin.

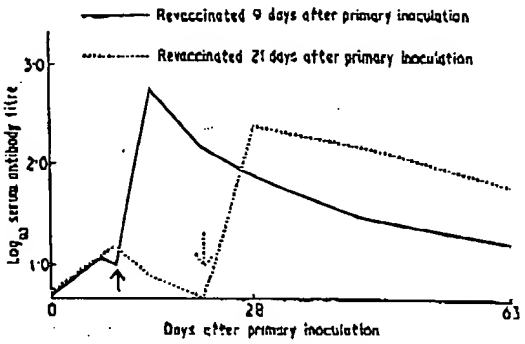

FIG. 7. Anamnestic response to a secondary inoculation of a type C saponin vaccine given at 9 or 21 days after primary vaccination with the same vaccine.

The purpose of this experiment was to ascertain whether the primary vaccination dose of a saponin vaccine sensitized the animal sufficiently to give a true secondary response. Two groups of 6 pigs were each given a dose of a type C saponin vaccine containing 200 c.f.u. total antigen. The type C antigen batch used for the DEAE-dextran vaccine was used in the saponin vaccine. One group then received a second dose at the height of the primary response 9 days after vaccination while the other group received a second dose 21 days after vaccination when there were no detectable serum neutralizing antibodies. Both groups showed a good anamnestic response (Fig. 7) and antibody concentrations decreased more slowly than after primary vaccination. The majority of the animals in both groups would have been immune to challenge 63 days after the primary dose as judged by serum antibody concentrations.

The class of antibody present in the 2 groups of pigs was examined 5 days after the second dose in the case of the group revaccinated 9 days after vaccination and 7 days after the second dose in the case of the group revaccinated 21 days after vaccination. The Sephadex G-200 profiles obtained are shown in Fig. 8. Giving a second injection at the height of the primary IgM response stimulated mainly further IgM production but some IgG was also produced. A second injection given 21 days after vaccination when no detectable

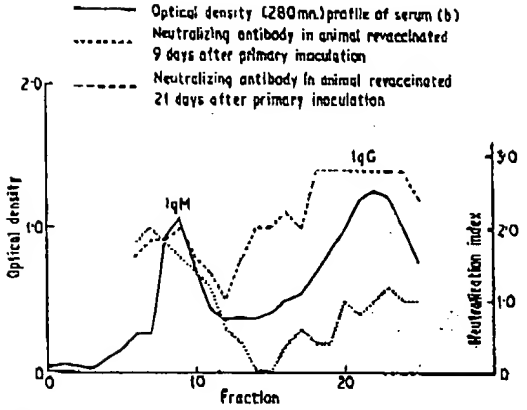

FIG. 8. Neutralizing antibody levels, expressed as the 'neutralization index' (NI) of Sephadex G-200 fractions of a serum collected 5 days after a second dose of a saponin vaccine given at 9 days after the primary dose and of a serum collected 7 days after a second dose given at 21 days after the primary dose.

circulating antibody was present resulted in the production of more IgM but also an appreciable amount of IgG. Even though circulating antibody of either class could not be detected a degree of immunological memory must have been established.

DISCUSSION

Pigs infected with FMD do not become 'carriers' (Burrows, 1968) and this ability of the pig to eliminate the virus and all further antigenic stimulation may in part explain why the duration of immunity in convalescent pigs is short. The high and long lasting serum antibody concentrations observed following vaccination with emulsion vaccines indicated that they possibly acted by providing frequent stimulation of the immune mechanism.

The response to DEAE-dextran vaccines was less good than the response to emulsion vaccines. The primary response was dose dependent, at least over the antigen ranges examined, but antibody concentrations were never great or very long lasting even though the change from IgM to IgG production did take place. Secondary vaccination resulted in a good anamnestic response, following which antibody concentrations decreased more slowly. Application of these vaccines in the field would require a second dose within 2 to 3 months of the initial vaccination to give a reasonable duration of immunity. The serological responses that we observed were similar to those found by Wittmann *et al.* (1970), *viz.* peak concentrations at 14 days after vaccination, followed by decreasing titres. Although Wittman *et al.* (1970) found that almost all their vaccinated pigs resisted a contact challenge with the homologous O_1 virus 12 weeks after vaccination, the titres had declined considerably by that time and the animals might not have been able to withstand challenge for much longer. Wittmann (1970) suggested that the adjuvant activity of DEAE-dextran could be due to a membrane effect on the immuno-competent cells. Increasing the dose of DEAE-dextran up to 500 mg. resulted in increased serum antibody concentrations, suggesting that increasing the dose of adjuvant stimulated more immuno-competent cells to become antibody producers.

It is also possible that saponin exerts its adjuvant activity through its effect on cell membranes but why saponin vaccines are less potent than DEAE-dextran vaccine is not clear. It is likely that in both cases all the antigen administered is quickly catabolized and removed. In fact a single dose of a saponin vaccine produced only a true primary IgM response but the animals became sensitized and gave an anamnestic response to a second injection. The degree of this response was less than with DEAE-dextran vaccines and more frequent doses at short intervals would be required to obtain an adequate level of immunity. This agrees with field experience (Lucam *et al.*, 1967) where repeated applications of these vaccines reduced the number of outbreaks of the disease in pigs.

These experiments indicate that the pig requires frequent stimulation with FMD antigen to produce reasonable concentrations of circulating antibody. It is postulated that a single dose of an emulsion vaccine provides this stimulus, resulting in long immunity, while a second or further doses of a DEAE-dextran vaccine are required to achieve a similar duration of immunity.

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